ELSEVIER

Archives of Gerontology and Geriatrics
25 (1997) 245–253

ARCHIVES OF GERONTOLOGY AND GERIATRICS

Effect of dietary restriction on serum antioxidant capacity in rats[1]

Guohua Cao [a,b,*], Ronald L. Prior [a], Richard G. Cutler [c], Byung P. Yu [d]

[a] *US Department of Agriculture, Agriculture Research Service, Jean Mayer Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA*
[b] *Nutritional Science Department, University of Connecticut, Storrs, CT 06269, USA*
[c] *Gerontology Research Center, National Institute on Aging, National Institutes of Health, 4940 Eastern Avenue, Baltimore, MD 21224, USA*
[d] *Department of Physiology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284, USA*

Received 30 January 1997; received in revised form 5 May 1997; accepted 6 May 1997

Abstract

The effects of dietary restriction on serum antioxidant capacities were studied in male Fischer 344 rats. Dietary restriction was started at the age of 6 weeks and consisted of 60% of the mean daily food intake of the ad libitum fed controls. They were killed at 7 and 18 months of age. The antioxidant capacities of whole serum and the non-protein fraction of serum were assessed using the oxygen radical absorbance capacity (ORAC) assay with a peroxyl radical generator. Rats that consumed a diet restricted by 40% in calories had significantly lower ORAC activities in whole serum and the non-protein fraction of serum. The decreased serum ORAC activity is seemingly an organism's physiologically appropriate response to reduced oxidative stress through a down-regulation mechanism. © 1997 Elsevier Science Ireland Ltd.

Keywords: Free radical; Antioxidant capacity; Dietary restriction; Rats

* Corresponding author. Tel.: +1 617 5563141; fax: +1 617 5563299.

[1] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

0167-4943/97/$17.00 © 1997 Elsevier Science Ireland Ltd. All rights reserved.
PII S0167-4943(97)00014-9

PM3006722760

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

1. Introduction

Chronic dietary restriction (DR) without essential nutrient deficiency is recognized as the most effective manipulation by which to extend the life span and retard the aging process in laboratory rodents and other short-lived species (Yu, 1996). Intensive research in the last several years has produced various plausible hypotheses for the mechanisms underlying the life-span extending effects of DR in rodents. One of the most exciting of these theories is the oxidative stress theory of aging (Yu, 1996; Yu and Yang, 1996), which provides the most convincing mechanistic explanation for both aging and age-related pathogenesis (Harman, 1993). According to this theory, both biological and pathologic aging processes are considered consequences of an increased susceptibility to damage from free radical-mediated oxidative stress. The anti-aging effect of DR could result from reduced reactive species (RS) production and/or enhanced defenses, which include enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX); macromolecules such as albumin, ceruloplasmin, and ferritin; and an array of micromolecules, including ascorbic acid, α-tocopherol, β-carotene, ubiquinol-10, glutathione (GSH), methionine, uric acid, and bilirubin (Yu, 1994).

Recent investigations clearly show that DR has the ability to modulate the production of various RS in rodents (Koizumi et al., 1987; Laganiere and Yu, 1987; Lee and Yu, 1990; Matsuo et al., 1993). It reduces the production of superoxide and hydroxyl radicals (Lee and Yu, 1990), and inhibits lipid peroxidation (Koizumi et al., 1987; Laganiere and Yu, 1987; Matsuo et al., 1993) and DNA damage (Chung et al., 1992). DR is also shown to affect antioxidant enzymes in diverse ways, including through its ability to increase SOD, CAT, and GPX activity and expression in liver (Koizumi et al., 1987; Laganiere and Yu, 1989b; Lammi-Keefe et al., 1984; Lee and Yu, 1990; Rao et al., 1990), and attenuate the CAT and GPX activity in skeletal muscle (Luhtala et al., 1994). However, the effect of DR on macro- and micromolecule antioxidant compounds is virtually unexplored. The objective of this study, therefore, is to initiate an investigation on the effect of DR on the serum antioxidant status using the oxygen radical absorbance capacity (ORAC) assay with a peroxyl radical ($ROO^{\cdot}$) generator (Cao et al., 1993).

2. Materials and methods

2.1. Chemicals

Porphyridium cruentum β-phycoerythrin (β-PE) was purchased from Sigma, St. Louis, MO. The β-PE that was used in these experiments usually lost more than 90% of its fluorescence within 30 min in the presence of 4 mmol/l of 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH). AAPH was obtained from Polyscience, Warrington, PA. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich, Milwaukee, WI.

PM3006722761

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

2.2. Animals and diet

The use of animals was conducted in compliance with all applicable laws and regulations as well as the principles expressed in the National Institutes of Health, USPHS, Guide for the Care and Use of Laboratory Animals.

Specific-pathogen free male Fischer 344 rats (4 weeks of age) were purchased from Charles River Laboratories, Kingston, NY. Upon receipt, rats were transferred into a barrier facility at the University of Texas Health Science Center at San Antonio, TX. They were housed individually in plastic cages with wire mesh floors. The rats were acclimatized for the first 2 weeks; DR was then implemented as described previously (Yu et al., 1985). Briefly, at 6 weeks of age, rats in the restricted group were fed 60% of the mean daily food intake of the rats fed ad libitum. The diet (Diet A) of the rats fed ad libitum and the diet (Diet B) of the restricted rats were basically the same, as prepared by the Purina Co. (Purina Test Chow, Richmond, IN), except for the concentrations of the mineral and vitamin mixes. Diet B provided the DR rats the same daily intake of vitamins and minerals as the mean intake of the rats fed ad libitum. The composition of the diets is presented in Table 1.

2.3. Blood sample preparation

Rats were killed by decapitation at 7 and 18 months of age. Serum samples were collected and stored at −80°C until analyzed. Both serum and serum non-protein fractions were used in the ORAC assay. For preparation of serum non-protein

Table 1
Composition of rat diets

Component	Diet A	Diet B
Casein (vitamin-free)	21	21
Sucrose	15	15
Dextrin	43.65	39.55
Corn oil	10	10
Ralston Purina mineral mix[1]	5	7.64
Ralston Purina vitamin mix[2]	2	3.33
Solka-Floc	3	3
D,L-methionine	0.15	0.15
Choline chloride	0.2	0.33

[a] Ralston Purina mineral mix has the following composition expressed in percent: $(NH_4)_6Mo_7O_2 \cdot 4H_2O$, 0.003; $CaCO_3$, 28.79; $CaHPO_4 \cdot 2H_2O$, 14.8; K_2HPO_4, 20.5; NaCl, 9.5; $NaH_2PO_4 \cdot H_2O$, 11.8; $MgSO_4 \cdot 7H_2O$, 13.5; $MnSO_4 \cdot H_2O$, 0.4; $FeC_6H_5O_7 \cdot 5H_2O$, 0.72; $CuSO_4 \cdot 5H_2O$, 0.12; KI, 0.0015; $ZnCl_2$, 0.0855; Na_2SeO_3, 0.001; $CrCl_3 \cdot 6H_2O$, 0.031; $CoCl_2 \cdot 6H_2O$, 0.026; NaF, 0.022.

[b] Ralston Purina vitamin mix has the following composition expressed in percent: thiamin hydrochloride, 0.1; riboflavin, 0.1; niacin, 0.45; pyridoxine hydrochloride, 0.1; D-calcium pantothenate, 0.3; folic acid, 0.02; D-biotin, 0.002; I-inositol, 1.0; vitamin B-12 (0.1% trituration; 1 g = 1 mg B-12), 0.1; retinal acetate, 0.4; ergocalciferol, 0.026; D,L-α-tocopherol acetate, 1.0; menadione sodium bisulfate, 0.1; sucrose, 96.302.

PM3006722762

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

fraction, the serum was diluted with saturated ammonium sulfate (1:4, v/v) and kept in ice water for 20 min. The samples were then centrifuged at 100 000 × g for 10 min at 4°C, and the supernatants were removed as the serum non-protein fractions for ORAC assay.

2.4. ORAC assay of serum and the serum fractions

The serum antioxidant capacity was determined by the ORAC assay. Briefly, in the final assay mixture (2 ml total volume), β-PE (3.34×10^{-8} mol/l) was used as the target of free radical attack, AAPH (4×10^{-3} mol/l) as the peroxyl radical ($ROO^{\cdot}$) generator, and Trolox (1 μmol/l, final concentration) as the control standard. Phosphate buffer (7.5×10^{-2} mol/l, pH 7.0) was used to prepare all the reagents. Following the addition of AAPH, the assay mixture was incubated at 37°C. Fluorescence of β-PE was measured every 5 min at an emission wavelength of 565 nm and excitation of 540 nm, using a fluorescence spectrophotometer, until zero fluorescence occurred. When a serum non-protein fraction was analyzed, ammonium sulfate was also used in the blank and standard. All fluorescent measurements were expressed relative to the initial reading. Final results were calculated using the differences of areas under the β-PE decay curves between the blank and a sample, and expressed as mmol/l Trolox equivalents (Cao et al., 1993, 1995). The ORAC activity of the protein fraction of a serum sample was calculated by subtracting the ORAC activity of the non-protein fraction of the serum from that of the whole serum.

2.5. Statistical analysis

The effects of age and DR, as well as their interaction, on serum ORAC activities were analyzed by two-way analysis of variance (ANOVA) using Systat (Systat, Evanston, IL). Multiple pairwise comparisons were evaluated by Tukey's honestly significant difference test used in Systat (Systat, Evanston, IL). The differences between two groups were analyzed by Student's two-sample t-test. Differences at $P < 0.05$ were considered significant. Data are presented as mean ± S.E.M.

3. Results and discussion

The effects of DR and age on the rat serum ORAC are shown in Fig. 1. Results of ANOVA showed that DR in rats significantly reduced the ORAC activities in the whole serum, and in the serum fraction as well [whole serum: $F(1,16) = 12.9$, $P = 0.002$; serum non-protein fraction: $F(1,15) = 17.0$, $P = 0.001$; serum protein fraction, $F(1,15) = 8.5$, $P = 0.01$]. A two-sample t-test also substantiated that the whole serum ORAC and serum non-protein ORAC activities were significantly reduced in both 7-month and 18-month-old DR animals compared to ad libitum controls. No significant age effect was observed in the ORAC activities of whole serum or serum fractions in these rats.

PM3006722763

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

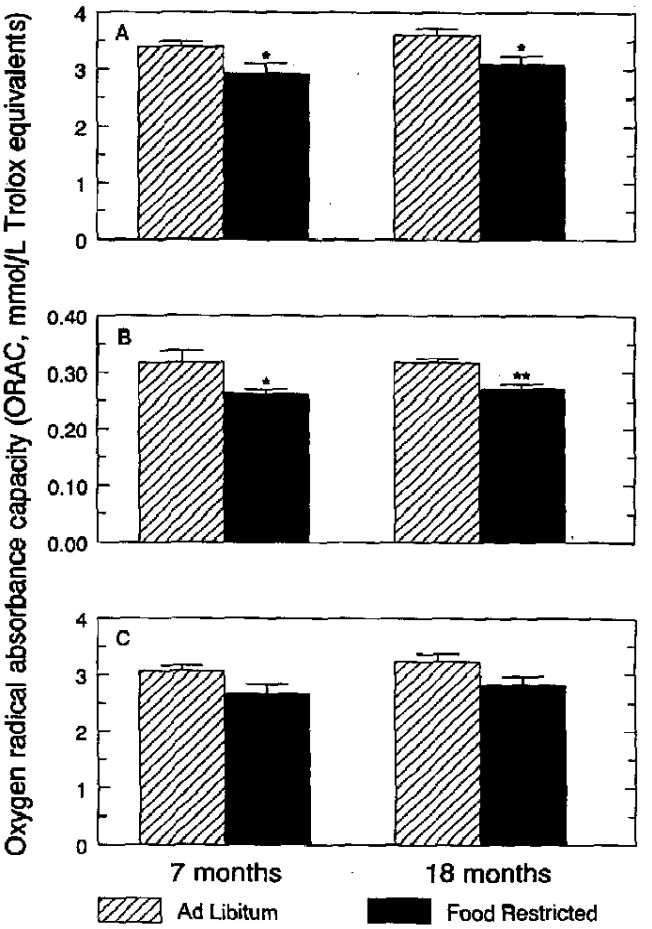

Fig. 1. Effects of dietary restriction on the oxygen radical absorbance capacity (ORAC) of rat serum and serum fraction. Dietary restriction was started at the age of 6 weeks and consisted of 60% of the mean daily food intake of the ad libitum fed controls. They were killed at 7 and 18 months of age. Each bar represents the mean ± S.E.M. for four or five animals. A, whole serum; B, serum non-protein fraction; C, serum protein fraction. Two-sample t-test (dietary-restricted group vs. group fed ad libitum): $*P \leq 0.05$, $**P < 0.01$. ANOVA showed a significant diet effect on ORAC not only in the whole serum ($P = 0.002$) and serum non-protein fraction ($P = 0.001$) but also in the serum protein fraction ($P = 0.011$).

The ORAC assay developed recently by Cao and coworkers (Cao et al., 1993, 1995) provides a unique and novel way to evaluate the antioxidant activities of various compounds and biological samples (Cao et al., 1996a,b,c; Miller et al., 1996; Pieri et al., 1994; Sharma et al., 1995; Yu et al., 1995; Wang et al., 1996). This method is superior to other similar methods (Wayner et al., 1985; Whitehead et al.,

PM3006722764

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

1992; Rice-Evans and Miller, 1994) because it uses an area-under-curve (AUC) technique, and thus combines both inhibition time and inhibition degree of free radical action by an antioxidant into a single quantity (Cao et al., 1995). The antioxidant capacity, measured using the ORAC assay with a peroxyl radical generator in this study, comes from all traditional antioxidants found in serum including ascorbic acid, α-tocopherol, β-carotene, GSH, bilirubin, uric acid, melatonin (Cao et al., 1993; Pieri et al., 1994), and flavonoids (Lin et al., 1996; Cao et al., 1997; Wang et al., 1997).

In view of the myriad of data available to support the oxidative stress theory of aging, one might expect an age-related decrease in serum antioxidant capacity. But, no change of serum ORAC with age was found in this rat study. This could be due to the age of animals used in the present study, since we did not maintain these rats on the diets until they were older than 18 months (after this age, control rats develop cancers more frequently). However, no significant age-related difference of serum antioxidant capacity was found when we used 6- and 22-month-old rats (Cao et al., 1996a). This is not surprising, because the levels of various antioxidants in rodent tissues are not uniformly affected by the aging process. For example, in rats, ascorbic acid levels show a decrease with aging in liver, lung and eye lens, but show no change in plasma, heart, kidney and brain (Rikans and Moore, 1988). Glutathione levels show no change with aging in plasma, liver, lung, heart, brain and eye lens, but show an increase in kidney (Rikans and Moore, 1988). In mice, ascorbic acid levels in eye lens and kidney show a decrease with aging, but no change in plasma and liver (Taylor et al., 1995a). Therefore, the aging process may result from accumulated oxidative damage not reflected by increased free radical production and/or decreased antioxidant levels in the body. In fact, the formation of hydroxyl radicals and hydrogen peroxide in the liver microsomes of rats is shown to decrease with age (Lee and Yu, 1990).

The decreased ORAC activity of whole serum and serum fractions in the DR rats is not unexpected because the responses of individual antioxidants to various effectors or stimuli, like aging processes, are known to be tissue-specific, not uniform or global (Rikans and Moore, 1988; Taylor et al., 1995a). Also, our results are consistent with reports that show a decrease of serum tocopherol (Laganiere and Yu, 1989a) and ferritin (Choi and Yu, 1994) levels in DR animals. Our results also support the recent findings of Taylor et al. (1995a,b), who found that mice that consumed a 40% caloric-restricted diet have lower ascorbate concentrations in plasma, liver, and kidney; yet, these DR mice show significantly delayed progression of cataract.

Why serum antioxidant activity measured by ORAC assay is lower in DR animals is an interesting and important question. Although a firm answer is not readily available, several possibilities exist. Firstly, the lower activity could be related to reduced antioxidant intake and absorption. But, this scenario is unlikely, because the amounts of dietary vitamins and minerals for DR rats are equivalent to those received by the controls fed ad libitum, if normalized on the basis of body weight. Also, gut absorptive activity is expected to be similar or increase under DR conditions, as indicated by enhanced pancreatic function (Chu et al., 1991) and

PM3006722765

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

vitamin A absorption (Hollander et al., 1986). Another possibility is that reduced serum ORAC is a result of the weakened antioxidant defenses in tissues of DR rats. This, however, is also unlikely because DR is shown to enhance the overall antioxidant defense system (Lammi-Keefe et al., 1984; Koizumi et al., 1987; Laganiere and Yu, 1987, 1989b; Lee and Yu, 1990; Rao et al., 1990; Chung et al., 1992; Matsuo et al., 1993; Choi and Yu, 1994).

A possible, but unconfirmed, answer may be found in the lower serum uric acid levels in DR rats, compared to their ad libitum-fed counterparts. Considering the higher incidence of nephropathic conditions (even as young as 6-months-old) in ad libitum-fed rats, which would cause higher serum uric acid levels, DR rats would then be expected to have reduced levels of this important serum antioxidant. However, as with other similar methods, the ORAC assay does not provide information on individual antioxidants in serum. Thus, the reduced specific antioxidant component or components measured by the ORAC assay in the serum of DR rats must await further exploration.

The decreased synthesis or production, and increased catabolism, of some specific antioxidants could also be an explanation for the lower ORAC activity in the DR rats. A logical speculation would be that the synthesis or production of some specific antioxidants is driven by RS, but suppressed by DR. Life-span extension by DR is known to depend on the chronic restriction of energy intake with the extent of life-span extension being directly related to the severity of DR (Weindruch and Walford, 1988). When the chronic restriction of energy intake reduces RS production (Laganiere and Yu, 1989b; Yu, 1994), some antioxidants like ascorbic acid may function as an energy source rather than a defense against oxidative stress (Taylor et al., 1995a).

Based on emerging evidence, the responses of antioxidant defenses to oxidative stress should not be considered globally, as many once thought, but rather be viewed as tissue- and antioxidant-specific. Thus, the lower ORAC activity observed in the serum of DR animals should not be taken to necessarily indicate lower overall antioxidant levels induced by DR. What the results of this present study seem to suggest is that the reduction of serum antioxidant status by DR is an organism's physiologically appropriate response to reduced oxidative stress in the serum through a down-regulation mechanism.

Acknowledgements

This work was supported in part by a grant to B.P.Y. from the National Institute on Aging (AGO1188).

References

Cao, G., Alessio, H.M., Cutler, R.G., 1993. Oxygen-radical absorbance capacity assay for antioxidants. Free Rad. Biol. Med. 14, 303–311.

PM3006722766

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

Cao, G., Verdon, C.P., Wu, A.H.B., Wang, H., Prior, R.L., 1995. Automated oxygen radical absorbance capacity assay using the COBAS FARA II. Clin. Chem. 41, 1738–1744.

Cao, G., Giovanoni, M., Prior, R.L., 1996a. Antioxidant capacity decreases during growth but not aging in rat serum and brain. Arch. Gerontol. Geriatr. 22, 27–37.

Cao, G., Giovanoni, M., Prior, R.L., 1996b. Antioxidant capacity in different tissues of young and old rats. Proc. Soc. Exp. Biol. Med. 211, 359–365.

Cao, G., Sofic, E., Prior, R.L., 1996c. Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 44, 3426–3431.

Cao, G., Sofic, E., Prior, R.L., 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Rad. Biol. Med. 22, 749–760.

Choi, J.H., Yu, B.P., 1994. Modulation of age-related alteration of iron, ferritin and lipid peroxidation in rat serum. Age 17, 93–97.

Chu, K.U., Ishizuka, J., Poston, G.J., Townsend, C.M., Greeley, G.H., Yu, B.P., Thompson, J.C., 1991. A change of the endocrine pancreatic function in short-term diet restriction. Nutrition 7, 425–429.

Chung, M.H., Kasai, H., Nishimura, S., Yu, B.P., 1992. Protection of DNA by dietary restriction. Free Rad. Biol. Med. 12, 523–525.

Harman, D., 1993. Free radicals and age-related diseases. In: Yu, B.P. (Ed.), Free Radicals in Aging. CRC Press, Boca Raton, pp. 206–222.

Hollander, D., Dadufalza, V., Weindruch, R., Walford, R.L., 1986. Influence of life-prolonging dietary restriction on intestinal vitamin A absorption in mice. Age 9, 59–60.

Koizumi, A., Weindruch, R., Walford, R.L., 1987. Influences of dietary restriction and age on liver enzyme activities and lipid peroxidation in mice. J. Nutr. 117, 361–367.

Laganiere, S., Yu, B.P., 1987. Anti-lipoperoxidation action of caloric restriction. Biochem. Biophys. Res. Commun. 145, 1185–1191.

Laganiere, S., Yu, B.P., 1989a. Effect of chronic caloric restriction in aging rats. I. Liver subcellular membranes. Mech. Ageing Dev. 48, 207–219.

Laganiere, S., Yu, B.P., 1989b. Effect of chronic caloric restriction in aging rats. II. Liver cytosolic antioxidants and related enzymes. Mech. Ageing. Dev. 48, 221–230.

Lammi-Keefe, C.J., Swan, P.B., Hegarty, P.V.J., 1984. Effect of level of dietary protein and total or partial starvation on catalase and superoxide dismutase activity in cardiac and skeletal muscles in young rat. J. Nutr. 114, 2235–2240.

Lee, D.W., Yu, B.P., 1990. Modulation of free radicals and superoxide dismutase by age and dietary restriction. Aging 2, 357–362.

Lin, Y.L., Juan, I.M., Chen, Y.L., Liang, Y.C., Lin, J.K., 1996. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. J Agric. Food Chem. 44, 1387–1394.

Luhtala, T.A., Roecker, E.B., Pugh, T., Feuers, R.J., Weindruch, R., 1994. Dietary restriction attenuates age-related increases in rat skeletal muscle antioxidant enzyme activities. J. Gerontol. Biol. Sci. 49, B231–B238.

Matsuo, M., Gomi, F., Kuramoto, K., Sagai, M., 1993. Caloric restriction suppresses age-dependent increases in the respired rates of hydrocarbon from rat. J. Gerontol. Biol. Sci. 48, B133–B138.

Miller, J.W., Selhub, J., Joseph, J.A., 1996. Oxidative damage caused by free radicals produced during catecholamine autoxidation: protective effects of *O*-methylation and melatonin. Free Rad. Biol. Med. 21, 241–249.

Pieri, C., Marra, M., Moroni, F., Recchioni, R., Marcheselli, F., 1994. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci. 55, PL271–PL276.

Rao, G., Xia, E., Nadakavukaren, M.J., Richardson, A., 1990. Effect of dietary restriction on the age-dependent changes in the expression of antioxidant enzymes in rat liver. J. Nutr. 120, 602–609.

Rice-Evans, C., Miller, N.J., 1994. Total antioxidant status in plasma and body fluids. Methods Enzymol. 234, 279–293.

Rikans, L.E., Moore, D.R., 1988. Effect of aging on aqueous-phase antioxidants in tissues of male Fischer rats. Biochim. Biophys. Acta 966, 269–275.

Sharma, H.M., Hanna, A.N., Kauffman, E.M., Newman, H.A.I., 1995. Effect of herbal mixture *student rasayana* on lipoxygenase activity and lipid peroxidation. Free Rad. Biol. Med. 18, 687–697.

PM3006722767

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001

Taylor, A., Lipman, R.D., Jahngen-Hodge, J., Palmer, V.J., Smith, D.E., Padhye, N., Dallal, G.E., Cyr, D.E., Laxman, E., Shepard, D., Morrow, F., Salomon, R., Perrone, G., Asmundsson, G., Meydani, M., Blumberg, J., Mune, M., Harrison, D.E., Archer, J.R., Shigenaga, M., 1995a. Dietary calorie restriction in the Emory mouse: effects on lifespan, eye lens cataract prevalence and progression, glucose and glycohemoglobin, tail collagen breaktime, DNA and RNA oxidation, skin integrity, fecundity, and cancer. Mech. Ageing Dev. 79, 33–57.

Taylor, A., Jahngen-Hodge, J., Smith, D.E., Palmer, V.J., Dallal, G.E., Lipman, R.D., Padhye, N., Frei, B., 1995b. Dietary restriction delays cataract and reduces ascorbate levels in Emory mouse. Exp. Eye Res. 61, 55–62.

Wang, H., Cao, G., Prior, R.L., 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44, 701–705.

Wang, H., Cao, G., Prior, R.L., 1997. The oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem. 45, 304–309.

Wayner, D.D.M., Burton, G.W., Ingold, K.U., Locke, S., 1985. Quantitative measurement of the total peroxyl radical-trapping antioxidant capacity of human blood plasma by controlled peroxidation. FEBS Lett. 187, 33–37.

Weindruch, R., Walford, R.L., 1988. The Retardation of Aging and Disease by Dietary Restriction. Charles C Thomas, Springfield, IL.

Whitehead, T.P., Thorpe, G.H.G., Maxwell, S.R.J., 1992. Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. Anal. Chim. Acta 266, 265–277.

Yu, B.P., Masoro, E.J., McMahan, C.A., 1985. Nutritional influences on aging of Fischer 344 rats: I. Physiological, metabolic and longevity characteristics. J. Gerontol. 40, 657–670.

Yu, B.P., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74, 139–162.

Yu, J., Fox, J.G., Blanco, M.C., Yan, L., Correa, P., Russel, R.M., 1995. Long-term supplementation of canthaxanthin does not inhibit gastric epithelial cell proliferation in *Helicobacter mustelae*-infected ferrets. J. Nutr. 125, 2493–2500.

Yu, B.P., 1996. Aging and oxidative stress: modulation by dietary restriction. Free Rad. Biol. Med. 21, 651–668.

Yu, B.P., Yang, R., 1996. Critical evaluation of the free radical theory of aging: a proposal of oxidative stress hypothesis. Ann. N.Y. Acad. Sci. 786, 1–11.

PM3006722768

Source: https://www.industrydocuments.ucsf.edu/docs/xqbj0001